

PATENT
Docket No. 21829/81 (EBC-006)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Fan et al.

Serial No. : 09/879,248

Cnfrm. No. : 4829

Filed : June 12, 2001

For : HYPERSENSITIVE RESPONSE ELICITING
DOMAINS AND USE THEREOFExaminer:
L. MayesArt Unit:
1653

DECLARATION OF ZHONG-MIN WEI UNDER 37 C.F.R. § 1.132

Mail Stop:
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, ZHONG-MIN WEI, pursuant of 37 C.F.R. § 1.132, declare:

1. I received a B.S. degree in Biology from Zhejiang University, Zhejiang, China in 1982, an M.S. degree in Plant Pathology from Nanjing Agricultural University, Nanjing, China in 1984, and a Ph.D. degree in Molecular Biology from Nanjing Agricultural University and Academy of Science, Shanghai, China in 1987.
2. I am currently employed as Chief Scientific Officer and Vice President of Research and Development at EDEN Bioscience Corporation in Bothell, Washington.
3. I am a co-inventor of the above-identified application.
4. I am presenting this declaration to show what domains are present in the protein fragment spanning amino acid sequences 1 through 218 of the amino acid sequence from the hypersensitive response elicitor HrpN from *Erwinia amylovora*.
5. The present invention is directed to the discovery that hypersensitive response eliciting domains include two subunits. The first subunit, the acidic portion, has at least 10 amino acids and a pI below 5. This acidic portion has a secondary structure in the form of a beta-sheet, a beta-turn, or an unordered form. The second subunit also has at least 10 amino acids and a secondary structure in the form of a stable alpha-helix. Neither the acid portion nor the alpha-helix subunit is independently sufficient to elicit the hypersensitive

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response in plants. Both subunits must be present for a hypersensitive response eliciting domain to elicit the hypersensitive response in plants.

6. HrpN, from *Erwinia amylovora*, is a hypersensitive response elicitor protein of 403 amino acids in length. Two hypersensitive response eliciting domains, as described above in paragraph 5, were identified within the native HrpN protein. The hypersensitive response eliciting domains span from amino acid 32 through 74 and from amino acid 157 through 180.

7. A protein comprising amino acid sequence 1 through 218 of the amino acid sequence from the hypersensitive response elicitor HrpN from *Erwinia amylovora* would simply represent a fragment of the native full-length HrpN protein. The HrpN fragment would comprise the two identified hypersensitive response eliciting domains (amino acid sequence 32-74, "first domain" and amino acid sequence 157-180, "second domain"), as well as the native flanking amino acid sequence 1 through 31, immediately preceding the first domain, amino acid sequence 75 through 156, immediately following the first domain and immediately preceding the second domain, and amino acid sequence 181 through 218, immediately following the second domain.

8. The hypersensitive response eliciting domains present in the protein comprising amino acid sequence 1 through 218 of the amino acid sequence from the hypersensitive response elicitor HrpN, are in a native form. The domains are present amongst the native flanking amino acid sequences, in their native orientation, such that would be found in the naturally occurring HrpN elicitor protein from *Erwinia amylovora*.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: June 23/2003

Zhongmin Wei
Zhong-Min Wei